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THE LOCATION OF CARDIAC VAGAL PREGANGLIONIC MOTONEURONES IN THE MEDULLA OF THE CAT

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SUMMARY

1. Electrophysiological techniques have been used to locate the origin of preganglionic vagal motoneurones supplying the heart of the cat.

2. The right cardiac vagal branches were identified anatomically and their ability to slow the heart was assessed by electrical stimulation. Control experiments revealed that contamination of cardiac branches by bronchomotor and oesophageal efferent fibres was likely to be small.

3. Fifty-seven neurones in the medulla were activated antidromically on stimulating the cardiac branches at up to 5 times the threshold for cardiac slowing. They had axons with conduction velocities between 3 and 15 m/sec, corresponding to B fibres.

4. None of these were located in the region of the dorsal motor nucleus of the vagus, in spite of repeated sampling there, but all were located in the region of the nucleus ambiguus. Histological examination of marked neurones (forty-six of the fifty-seven neurones) revealed that they were associated with its principal column, rostral to the obex.

5. Sampling motoneurones of the dorsal motor nucleus revealed that most sent axons down the thoracic vagus below the cardiac branches. Only three of thirty-three could be activated antidromically by high intensity stimulation of the cardiac branches, but on the basis of their thresholds and conduction velocities, it is argued that they were unlikely to be cardio-inhibitory neurones.

6. It is concluded that preganglionic cardio-inhibitory neurones arise not in the dorsal motor nucleus, but in the principal column of the nucleus ambiguus.

INTRODUCTION

The site of origin of the preganglionic vagal fibres that slow the heart has been a matter of controversy for many years. The consensus of anatomical evidence, based almost exclusively on retrograde changes produced in medullary neurones after section of the various vagal branches,

has tended to favour the dorsal motor nucleus of the vagus close to the floor of the IVth ventricle (for references see Mitchell & Warwick, 1955). A notable exception to this is the observation of Szentagothai (1952) who made lesions in the medulla of cats and looked for axonal degeneration in the cervical and thoracic vagus and its branches. He stated that the cardiac efferent fibres originate in the rostral part of the nucleus ambiguous – the nucleus retrofacialis. Later, Calaresu & Cottle (1965) were able to find only occasional, sparse axonal degeneration in the vagal branches to the heart after destroying the dorsal motor nucleus.

Physiological evidence, based on stimulation of the medulla, has favoured either or both vagal nuclei (e.g. Miller & Bowman, 1915; Chiurugi & Mollica, 1954; Calaresu & Pearce, 1965; Gunn, Sevelius, Puiggari & Myers, 1968). However, two points must be borne in mind when interpreting these observations. Firstly, the dorsal motor nucleus is adjacent to the nucleus of the tractus solitarius, where baroreceptor and other 'depressor' afferents terminate, and stimulation there produces bradycardia; secondly, bradycardia will be produced by stimulation of not only cell bodies but also of motor axons or indeed any part of a reflex pathway exciting the final common pathway, the preganglionic vagal motoneurones. Indeed, axons from cells in the dorsal motor nucleus pass close to the nucleus ambiguus, and the reverse may also be the case (Cajal, 1909; Lawn, 1964). Thus stimulus spread over even short distances may give rise to quite misleading results.

The present study was undertaken in an attempt to define the site of cardiac vagal efferent neurones directly, using electrophysiological recording techniques. Use has been made of pontamine blue dye to mark the positions of neurones antidromically activated by stimulation of the cardiac vagal branches in anaesthetized cats. Preliminary observations have been reported to the Physiological Society (McAllen & Spyer, 1975).

METHODS

Experiments were performed on twenty-four female cats (wt. 2–3 kg), anaesthetized with either α-chloralose (70 mg/kg I.V., twenty-one cats) or a mixture of α-chloralose (35 mg/kg) and urethane (700 mg/kg I.V., three cats) after induction with ethyl chloride and ether. Supplementary doses of anaesthetic were given if and when necessary, either as a small dose of the original anaesthetic or by a small dose (6 mg) of sodium pentobarbitone. In all experiments the trachea was cannulated low in the neck, and cannulae placed in a femoral artery and vein for the measurement of arterial blood pressure and the administration of anaesthetic respectively.

The animal was then placed in a stereotaxic head holder and laid on its left side. The chest was opened between the right fourth and fifth ribs and the animal was then ventilated by means of a positive pressure respiratory pump (Harvard) connected also to an expiratory resistance of 0·5–1·5 cm H_2O to prevent complete collapse of the lungs. The ventilatory minute volume was adjusted to maintain the end-tidal

CO_2 at 3·5–4·5%, this being monitored continuously using a Beckman LB-1 CO_2 meter. Hourly arterial blood samples were taken from a cannula in the second femoral artery for blood gas analysis, and arterial HCO_3^- concentration was maintained at around 23 m-equiv/100 ml blood by I.V. injections of sodium bicarbonate solution. The animals' temperature was maintained between 37 and 38° C using an electric blanket controlled by a feed-back circuit.

Isolation and identification of cardiac branches

On opening the chest, the fourth and fifth ribs were retracted to expose the upper lobe of the right lung, which was then ligated and excised to expose the azygos vein. This was then tied and cut to expose the thoracic vagus. The vagus was then searched for branches coursing towards the heart. There was always at least one branch at the level of azygos vein (caudovagal branch) and one several cm rostral to this (craniovagal branch) although there were sometimes two or three of either (see Fig. 1). Each

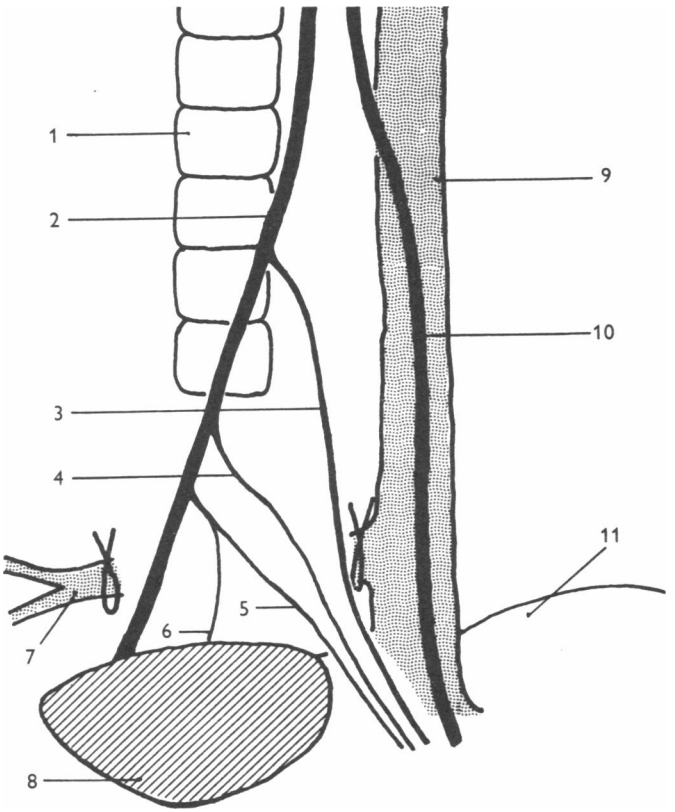

Fig. 1. Schematic diagram of dissection of right cardiac vagal branches. 1, trachea; 2, thoracic vagus; 3, craniovagal cardiac branch; 4, 5, caudovagal cardiac branches; 6, offshoot to lung; 7, divided azygos vein; 8, ligated root of upper lobe of lung; 9, superior vena cava; 10, phrenic nerve; 11, right atrium.

of these branches was then bluntly dissected free and placed, while still intact, on a pair of silver wire electrodes (separation 2–3 mm). The branch was then stimulated with rectangular pulses (2–3 V, 0·1 msec, 75 Hz) to assess whether it contained fibres which slowed the heart. If so, the threshold for this effect was measured. Thresholds thus established ranged from 0·1 to 2 V with a mean of 0·6 V (thirty-two branches). Such branches were carefully freed from offshoots that coursed towards the lungs (see Fig. 1). Before the recording experiment the branches were cut close to the heart and then mounted together on stimulating electrodes and covered with wisps of cotton-wool soaked in liquid paraffin. The threshold criterion used in recording experiments for assessing unitary responses was the highest threshold of the branches used.

Assessing the purity of cardiac branches

In three experiments respiratory airflow and pressure were recorded. Respiratory airflow was monitored using a Fleisch pneumotachograph attached to a Grass PT 5 A differential pressure transducer; respiratory pressure was recorded using a pressure transducer attached to the tracheal cannula. Oesophageal contractions were studied in two of these experiments by means of a saline-filled balloon attached to a pressure transducer which was advanced through the mouth to the oesophagus. These parameters together with arterial blood pressure and heart rate were displayed on a pen recorder.

In these experiments the cervical vagus, cardiac vagal branches (identified as above) and the thoracic vagus beneath the level of the azygos vein were stimulated. Changes in airway resistance were assessed by plotting respiratory pressure against respiratory flow using an $X-Y$ recorder, an increase in resistance producing an increased slope of the plot. Oesophageal contractions evoked pressure changes in the saline-filled balloon.

Recording experiments

In order to expose the medulla, the occipital bone was removed and the dura over the cerebellum excised along the mid line. The caudal portions of the cerebellum were removed by suction and the exposed floor of the IVth ventricle was kept moist with warm physiological saline or liquid paraffin. The activity of medullary neurones was recorded using techniques described in a previous paper (Lipski, McAllen & Spyer, 1975).

Recordings were made from the region of the two vagal motor nuclei (dorsal motor nucleus and nucleus ambiguus) throughout their rostro-caudal extent in the medulla during stimulation of the identified vagal cardiac branches. In a second series of experiments, cells in the dorsal motor nucleus were identified by their antidromic response to stimulation of the cervical vagus, and tested for their response to stimulation of the cardiac branches and of the thoracic vagus at a level below the azygos vein. Responsive neurones were marked by the expulsion of pontamine sky blue dye (Hellon, 1971). In some experiments phrenic nerve activity was recorded in the chest using a pair of silver wire electrodes and employing conventional recording techniques.

At the end of each experiment the brain was removed and fixed in 10% formol saline. Histological preparations and the mapping of responsive units are as described in a previous paper (Lipski *et al.* 1975).

RESULTS

Controls for purity of cardiac branches

Since the validity of our observations regarding the location of cardio-inhibitory neurones in the brain stem is dependent on the use of vagal branches containing a relatively pure population of cardiac efferent fibres,

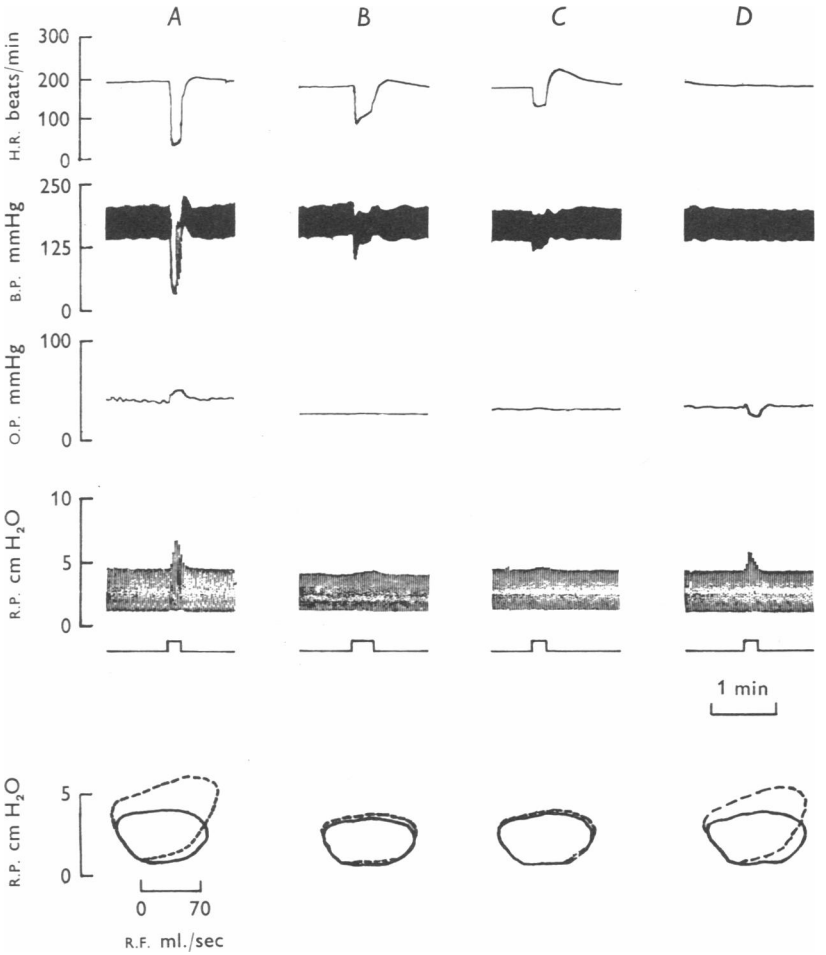

Fig. 2. Effects of stimulating various vagal branches on heart, bronchi and oesophagus. Traces from top: heart rate (H.R.), blood pressure (B.P.), oesophageal pressure (O.P.), tracheal pressure (R.P.), stimulus marker, $X-Y$ plot of tracheal pressure *vs.* respiratory airflow (R.F.) (interrupted line peak: response; continuous line: control). Effects of stimulation of (*A*) right cervical vagus (10 V, 75 Hz) (*B*) craniovagal cardiac branch (5 V, 75 Hz), (*C*) caudovagal cardiac branch (5 V, 75 Hz), and (*D*) thoracic vagus, caudal to azygos vein (10 V, 75 Hz).

a series of control experiments were done to assess the purity of cardiac vagal branches identified as described above. Three cats were thoracotomized as described in the Methods, and the thoracic vagal branches exposed. Measurements were made of lung resistance (three cats) and oesophageal contraction (two cats) in addition to heart rate, since we considered bronchoconstrictor and oesophageal efferents the most likely contaminants of presumed 'cardiac' vagal branches. Fig. 2 shows records

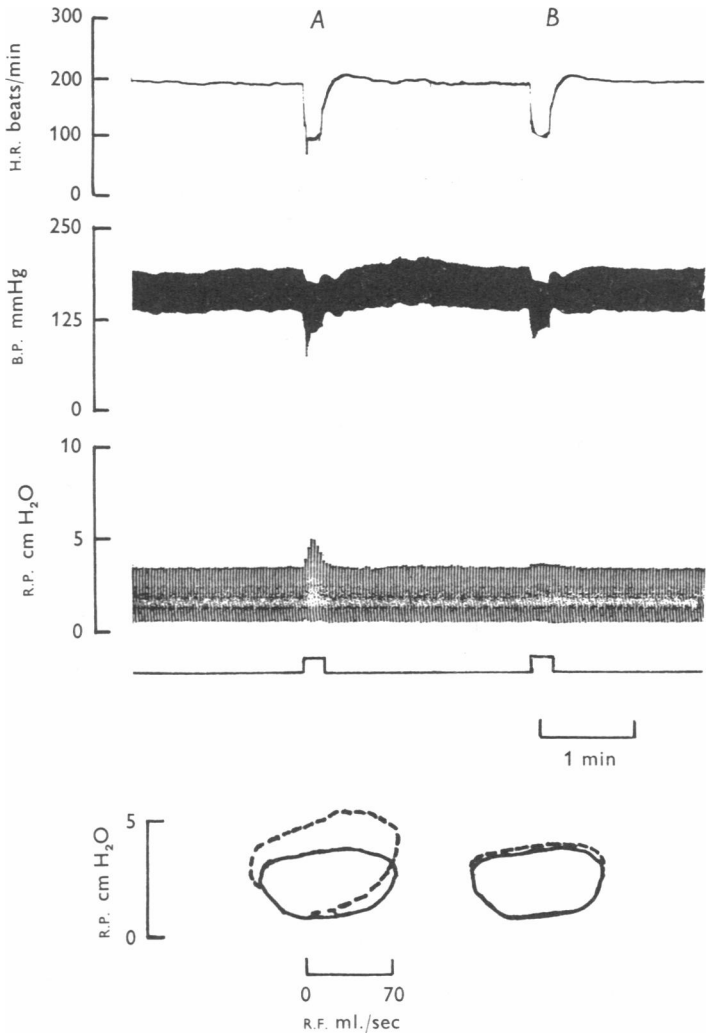

Fig. 3. Effects of stimulating right cervical vagus (*A*) before and (*B*) after cutting the thoracic vagus below cardiac branches (10 V, 75 Hz). Traces as in Fig. 2, but without oesophageal pressure.

from one such experiment although the others gave equivalent results. Stimulation of the cervical vagus (Fig. 2*A*) produced changes in heart rate and oesophageal contraction together with an increase in respiratory pressure. By plotting respiratory pressure against airflow, the increase in slope of the 'loop' seen during stimulation of the cervical vagus (lower trace, Fig. 2*A*) indicates an increase in airway resistance presumably due to bronchoconstriction. Stimulation of the cardiac branches (Fig. 2*B*, *C*) produced bradycardia. Although there were minimal changes in respiratory pressure, there were probably the passive effects of vascular congestion as they could be mimicked by clamping the pulmonary vein, and are unlikely to represent a change in airway resistance through bronchoconstriction as the pressure–flow plot did not noticeably alter in slope (bottom traces, Fig. 2*B*, *C*). No changes in the activity of the oesophagus were noted.

No vagal cardiac branches would seem to arise below the level of the azygos vein, since the fall in heart rate to stimulation of the cervical vagus was undiminished by sectioning the thoracic vagus at this level (Fig. 3*B*) and stimulation of peripheral end of the thoracic vagus gave no bradycardia (Fig. 2*D*). However, bronchoconstrictor fibres appear to branch from the vagus largely below this level (see Figs. 2*D* and 3*A*).

In summary we believe that the contamination of cardiac branches with bronchomotor and oesophageal efferent fibres, if present at all, was small and unlikely to affect the conclusions to be drawn from our observations.

Cardiac efferent neurones

Recordings were made from the medulla of cats in the region of both vagal motor nuclei. Units were sought that could be antidromically activated by stimulation of the cardiac vagal branches identified as above. Such units were found in the region of the nucleus ambiguus.

Cardiomotor neurones were identified primarily by the constant latency of their response to stimulation at 2 Hz. A signal averager greatly facilitated the search, but results were taken only from units checked for all-or-none responses and whose thresholds for activation could be measured. Fig. 4 shows a record obtained from such a neurone. This unit shows a clear inflexion on the rising phase of the spike, as has been described in many other neurones (Eccles, 1964). The second component, which would seem to correspond to the soma (SD) spike of Coombs, Curtis & Eccles (1957) could be induced to fail in a proportion of responses to repetitive stimuli (see Fig. 4*B*, *C*). Nine of the fifty-seven cardiomotor neurones studied showed clear two-component spikes (IS-SD spikes). Otherwise,

spikes were usually negative or negative–positive in configuration, although a small initial positive dip was sometimes seen (Fig. 4).

Under the conditions of our experiments in which the alveolar CO_2 was maintained within a range of 3·5–4·5 %, only sixteen neurones (28 % of the total) were spontaneously active, and then only when the animal showed signs of spontaneous breathing as assessed by the activity of neighbouring medullary respiratory neurones (eleven cases) or phrenic nerve activity (five cases). In two further cases, neurones not showing spontaneous

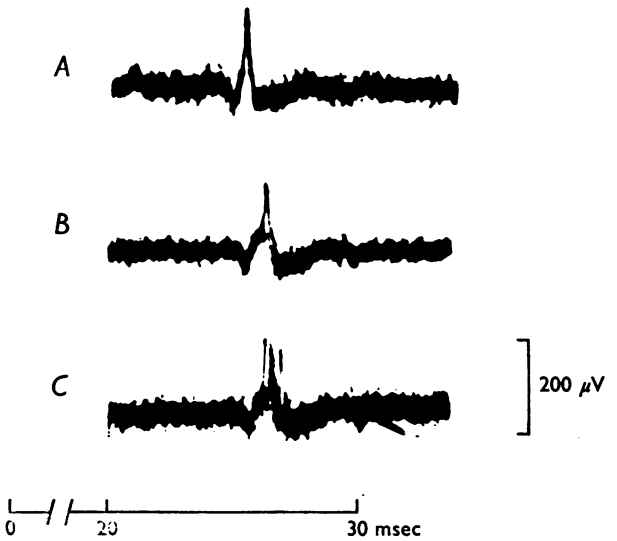

Fig. 4. Cardiac efferent unit: antidromic response to cardiac branch stimulation. Each trace shows approximately ten superimposed sweeps. *A*, stimulation at 1 Hz; *B*, immediately after raising stimulation frequency to 10 Hz; *C*, response to stimulation at 10 Hz after several seconds at this rate. Note expanded time scale.

activity were induced to fire if the alveolar CO_2 was raised above this level by underventilation. Unfortunately, the activity of respiratory (inspiratory) neurones in the vicinity often made the discrimination of the vagal efferent unit difficult, so experiments were carried under mild hyperventilation (alveolar CO_2 close to 3·5 %) to suppress this activity. When present, spontaneous spikes occurring at the appropriate time with respect to the stimulus could be seen to cancel the antidromically evoked spike by collision (see Fig. 5). Spontaneous activity, when present, generally occurred during expiration. This was true for all five spontaneously active cardiomotor units recorded in preparations in which phrenic nerve activity

was simultaneously recorded. In the unit illustrated in Fig. 6 cancellation of the antidromically evoked spike only occurs during expiration.

At stimulation frequencies greater than 2 Hz, units followed with an increased (by up to 10%) but steady, latency (Fig. 4*C*, *D*). The increased

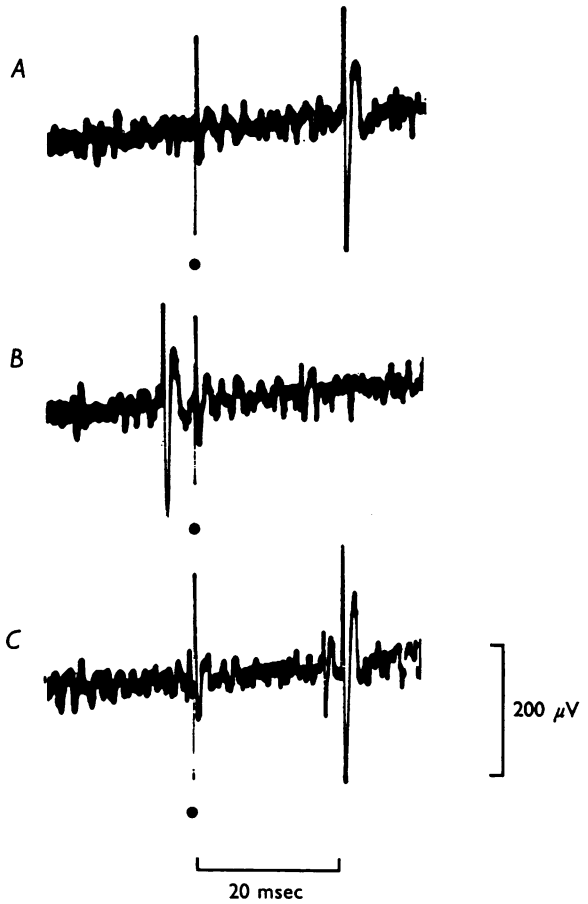

Fig. 5. Three successive sweeps of cardiac efferent unit activity during cardiac branch stimulation at 2 Hz (marked by artifacts and dots). In *B* a spontaneous spike occurring within the critical period cancels the normally occurring antidromic response (*A* and *C*). (Absolute refractory period of this unit less than 4 msec.)

latency appeared to be due not only to a delay in the invasion of the soma, but also to a decreased conduction velocity of the axon (Fig. 4*C*, *D*, cf. Bianchi, 1971). A qualitative assessment of this phenomenon indicates that the slowed conduction is partly due to relative refractoriness of the axon

(which lasts over 100 msec, Grundfest, 1939) from the previous spike, as it was also seen for the second response to a pair of stimuli. However, part is due to a cumulative effect that builds up during the tetanus, similar to that seen by Brown & Holmes (1955) for C fibres. Similarly, although of

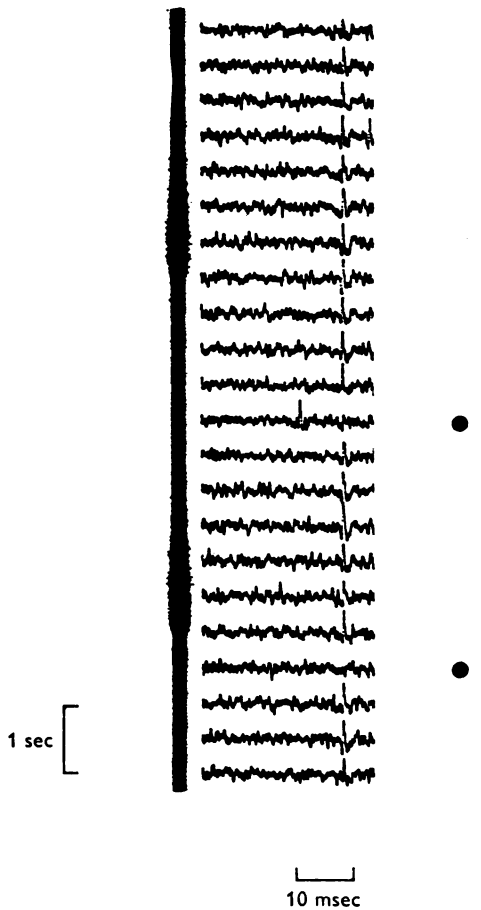

Fig. 6. Excerpt from a 'ladder' of successive responses to stimulation of cardiac branches at 2 Hz. Stimulus given at start of each horizontal trace. Vertical trace: phrenic nerve activity (direction, downwards). Dots mark two traces in which cancellation of the antidromic spike occurred.

fifteen units tested in detail, ten had an absolute refractory period less than 5 msec (mean 7·6 msec, range 3–26 msec), only two would follow several seconds of stimulation at 200 Hz (all followed 20 Hz, nine followed 100 Hz). However, since the open chest caused the thoracic vagus and cardiac branches to cool to approximately 30°C, and we consider this

likely to have exaggerated the above effects, we did not attempt to analyse them quantitatively.

Fig. 7*A* shows a histogram of the thresholds measured for cardiac efferent neurones expressed as a multiple of the threshold for cardiac slowing when the intact branch was stimulated. To minimize the problem of stimulus spread, we discarded those units with a threshold greater than

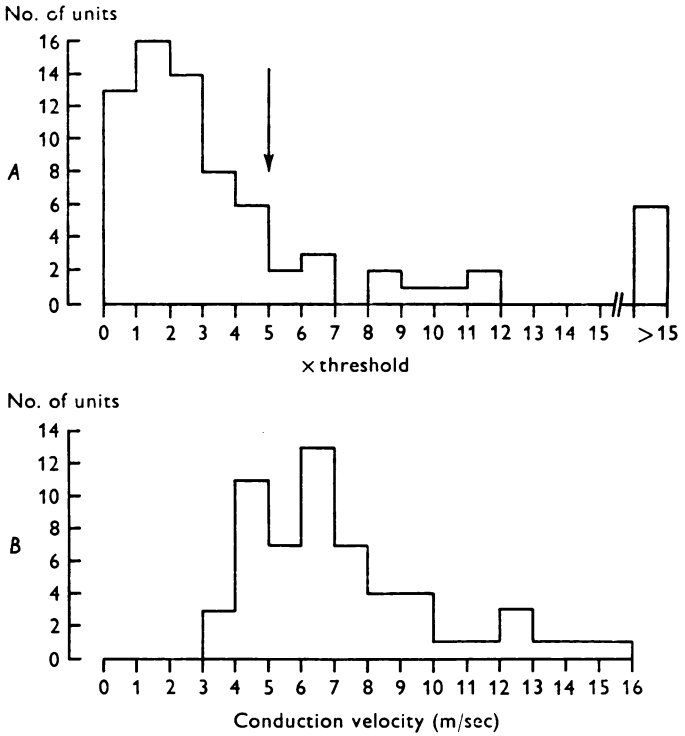

Fig. 7. *A*, histogram of number of medullary neurones responding antidromically to cardiac branch stimulation plotted against their threshold, expressed as a multiple of the threshold for cardiac slowing of the intact branch. Arrow denotes level above which units were discarded because of doubts about stimulus spread. *B*, histogram of conduction velocities of cardiac efferent units (those within the threshold criteria shown above). Uncorrected for cooling in chest (see text).

5 times that for cardiac slowing. The conduction velocities of the remaining fifty-seven neurones were calculated assuming an intracranial conduction distance of 1·5 cm beyond the jugular fossa. We estimate that cooling of the nerve in the thorax gave rise to a systematic underestimation of the true conduction velocity by approximately 10%. The uncorrected

conduction velocities are shown in Fig. 7*B*. Even allowing for this error, it is clear that they all fall within the range of B fibres, which have been shown to be responsible for the vagal bradycardia and negative inotropic effect on the atrium (Middleton, Middleton & Grundfest, 1950). They are also in agreement with the values obtained for presumed cardio-inhibitory fibres in the cat (Kunze, 1972) and dog (Jewett, 1964; Iriuchijima & Kumada, 1964).

Location

All presumed cardiac efferent neurones that fell within the criteria outlined above were restricted to the region of the nucleus ambiguus. Forty-six of these (81 %) were marked by deposition of pontamine blue, and their locations are shown in Fig. 8. The nucleus ambiguus in the cat appears very similar to that described in detail by Lawn (1966) for the rabbit. It is a sparse column of medium sized and large cells extending from about 2 mm caudal to 4 mm rostral to the obex, splitting in its more rostral part into a medial and a principal column, with a small dorsomedial group sometimes

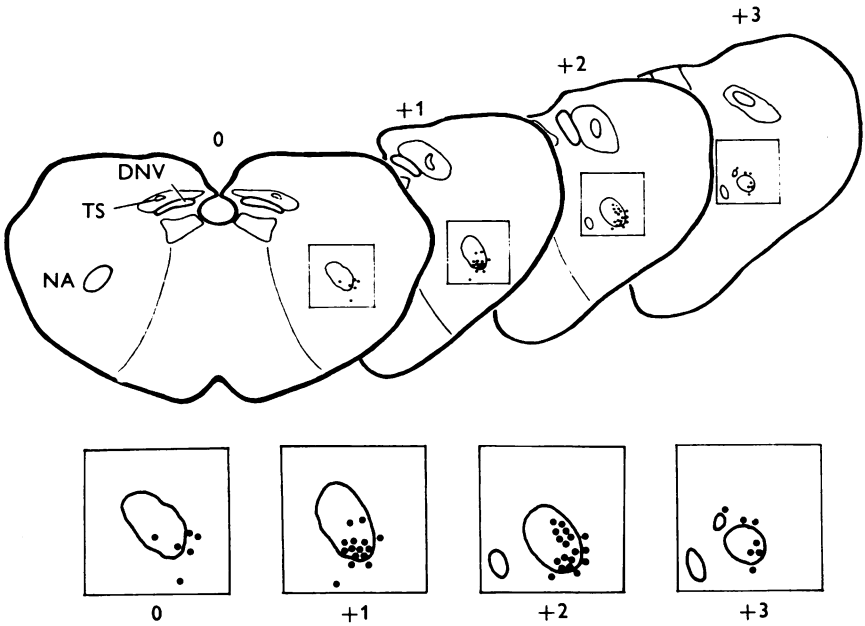

Fig. 8. Location. The positions of forty-six cardiac efferent neurones are shown on four standard sections of the medulla taken at obex level, and at 1 mm intervals rostrally. Inserts, 2 mm square, show details of their relation to the structure of the nucleus ambiguus. Abbreviations: TS, tractus solitarius; DNV, dorsal motor nucleus of the vagus; NA, nucleus ambiguus.

distinguishable from the main body of the principal column. No cardiomotor neurone was found further than 0·5 mm caudal to the obex. They were found extending in a column from obex level for about 3 mm rostrally, their greatest density being in the middle of this range. They can be seen most commonly in the lateral and ventrolateral aspects of the nucleus, and rostrally, they are clearly associated with the principal rather than the medial column. They do not appear to correspond well to the nucleus retrofacialis as we originally suggested (McAllen & Spyer, 1975).

Dorsal motor nucleus of the vagus

We did not find any cardiac efferent neurones that obeyed our criteria with the region of the dorsal motor nucleus of the vagus. However, a negative statement such as this begs the question of how thoroughly the area

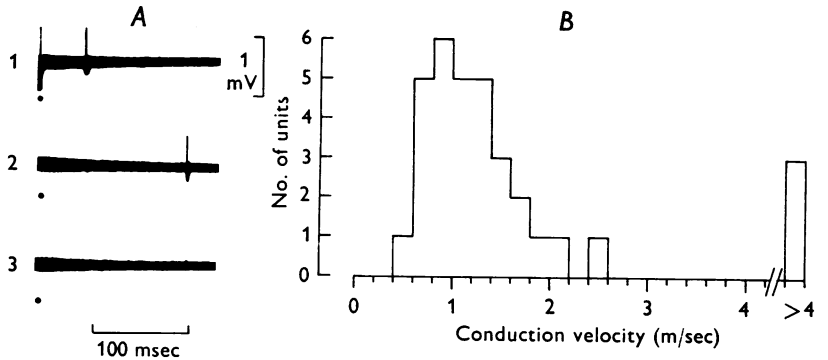

Fig. 9. Dorsal motor nucleus units. *A* show sets of ten superimposed sweeps of the dorsal motor nucleus of the vagus (DNV) unit responses to stimulation of cervical vagus (1); thoracic vagus below the azygos vein (2) and lack of response to cardiac branch stimulation (3). Stimuli marked by dots. *B* shows a histogram of the conduction velocities of thirty-three DNV units.

was explored. To add weight to this conclusion, a series of experiments were done in which we identified motoneurones throughout the extent of the dorsal motor nucleus of the vagus by their antidromic response to stimulation of the intact cervical vagus (cf. Fussey, Kidd & Whitwam. 1973). Units thus isolated were then tested for their response to stimulation of the cardiac branches and the remainder of the thoracic vagus. The data presented is for single units, but it is supported by extensive multiunit records. As can be seen from the calculated conduction velocities (Fig. 9*B*) they comprise a completely different population to the cardiomotor neurones described earlier. Almost all their axons fall in the C fibre range which does not include the cardio-inhibitory fibres (Middleton *et al.*

1950; R. M. McAllen & K. M. Spyer, unpublished observations). The majority of those tested (fifteen out of twenty) were also fired antidromically from the thoracic vagus, but not the cardiac branches (Fig. 9*A*). Three could be fired by high voltage stimulation of the cardiac branch (thresholds, 12, 20 and 22 times the thresholds for bradycardia on stimulating the intact cardiac branches, i.e. outside our criteria). In one case we are confident that this high stimulus intensity was not spreading to the rest of the thoracic vagus as stimulation of the thoracic vagus was ineffective. In all three cases the conduction velocity of the axon was in the C fibre range.

DISCUSSION

The results presented in the previous section have shown that there is a population of neurones in the nucleus ambiguus, extending rostrally from the level of the obex, which send fibres to the right cardiac branches of the thoracic vagus. The axons of these neurones are B fibres and the threshold for their activation is closely related to the intensity of stimulation required to produce bradycardia when the intact vagal branches were stimulated. These observations give rise to two main questions. Firstly, are the fibres, and consequently the antidromically identified neurones in the medulla, cardio-inhibitory in function? And secondly, are the units identified within the nucleus ambiguus cell bodies of cardiomotor neurones, or do they reflect the activity of fibres *en passant* from cells elsewhere?

We would like to consider that these neurones are cardiomotor in function. In support of this conclusion, we have provided evidence that cardio-inhibitory fibres of the right vagus course in the cardiac branches we identified, and that the contamination of these branches with other vagal efferent fibres was likely to be small. In this context only vagal efferent fibres are important; the admixture of sympathetic fibres is irrelevant since they do not originate in the brain, and the fact that the cardiac branches used in this study may contain a majority of afferent fibres (Agostoni, Chinnock, Daly & Murray, 1957) need not concern us except in the unlikely event of our recording from the intracranial part of an afferent fibre and mistaking this response for an antidromically activated motoneurone (see below). With respect to the non-cardiac vagal efferent fibres that we considered to be the most likely contaminants, we checked anatomically that the relevant branches coursed towards the heart and not to the lungs or oesophagus, and have shown functionally that they did not contain a significant proportion of either bronchial or oesophageal motor fibres. We did not investigate the possibility of their containing fibres controlling mucus secretion, although one would expect these to travel with the corresponding motor supply.

Whilst this evidence suggests strongly that we have been dealing with cardiac vagal efferent neurones, we have no means of distinguishing those with negative inotropic or dromotropic function from those with a negative chronotropic function, if, indeed, they have different preganglionic fibres, since they probably travel in the same branches. Furthermore it has been suggested that the dorsal motor nucleus contains neurones whose axons might be destined for the coronary vessels (Calaresu & Cottle, 1965) and this may account for our finding three C-fibre axons in the cardiac branches from cells in the dorsal motor nucleus.

Thus, we are led to the conclusion that no group of vagal efferent neurones other than cardio-inhibitory ones, is likely to have contributed significantly to the population of motoneurones described in this paper. But finally, even if only a proportion of the units antidromically activated from the cardiac branches is cardio-inhibitory in function, our conclusion is still valid. Middleton *et al.* (1950) have shown that cardio-inhibitory fibres are B-fibres. Our experiments have shown that efferent B-fibres in the cardiac branches do not originate in the dorsal motor nucleus, but only in the nucleus ambiguus: this must therefore also be true of cardio-inhibitory fibres.

The second point that needs to be answered is whether the units antidromically activated from the cardiac branches do indeed represent the activity of cell bodies, or merely of the axons of cells situated elsewhere. While it is not possible to answer this unequivocally, several pieces of evidence taken together provide support for our contention that our recordings were from somata. Firstly, a number of identified units had a clear inflexion on the rising phase of the spike potential, which has been seen in recordings from cell bodies elsewhere in the central nervous system (Eccles, 1964). Secondly, the activity of single cardiomotor neurones could usually be recorded over depths of 100 μm or more, and were found to be best recorded with low impedance micro-electrodes. Elsewhere we have argued that our recording techniques are unlikely to discriminate the activity of single fibres (Lipski *et al.* 1975; see also Salmoiraghi & Burns, 1960), and indeed in the present series of experiments all electrode penetrations that reached the nucleus ambiguus would have passed through the motor nerve tract of the vagus, but none of the antidromically activated units was found there. Further, no constant-latency response was found anywhere in the medulla with a latency corresponding to that of the population of afferent A-fibres in the caudovagal branch (Öberg & Thoren, 1973).

Together, these observations argue strongly that the cell bodies of vagal cardio-inhibitory neurones are located in the nucleus ambiguus in the cat's medulla. Although this contradicts the classical view, it is in keeping with

more recent evidence. The traditional histological methods (looking for chromatolytic neurones after section of various nerve branches) have yielded different results in different hands (see Mitchell & Warwick, 1955). Some of the disagreement may be due to genuine species differences, but this still would not explain different results on the same species. The variability of results from this method might be due in part to the problems of looking for changes in a small number of cells against the background of a complex surgical exposure, which might damage many other fibres; and probably part is a result of the subjectivity of assessment and variability of such changes (Brodal, 1969). The reverse method – making lesions in the brain and following the resultant wallerian degeneration in peripheral fibres – has provided weak or no support for the dorsal motor nucleus (Szentagothai, 1952; Calarescu & Cottle, 1965), but strong support for the nucleus ambiguus (Szentagothai, 1952). However, the degeneration seen by Szentagothai might equally have been due to interruption of the efferent fibre tract, leaving the question of the site of the cell bodies unanswered.

Similar problems exist for the interpretation of results of the other traditional technique, electrical stimulation. As mentioned earlier, the production of bradycardia by electrical stimulation of the medulla does not necessarily mean that cardio-inhibitory neurones were directly stimulated. In view of the large number of possibilities of obtaining a vagal bradycardia on central stimulation, one is perhaps justified in paying more attention to negative results. In this context, Calaresu & Pearce (1965) were unable to obtain bradycardia on stimulating within the dorsal motor nucleus in cats, a finding confirmed by Gunn *et al.* (1968), who additionally showed that the nucleus ambiguus gave positive results. In the dog, however, both loci were effective (Gunn *et al.* 1968). Surprisingly, in other experiments Calaresu has been able to produce bradycardia on stimulating the dorsal motor nucleus (Thomas & Calaresu, 1974) although in this case the response was markedly attenuated by sodium pentobarbitone, while the heart rate response to stimulating the nucleus ambiguus remained unaltered.

More convincing evidence that cardio-inhibitory neurones are not in the dorsal motor nucleus in the cat has been provided by the ablation experiments of Kerr (1967, 1969) and Borison and Domjan (1970). Kerr (1967, 1969) found that chronic unilateral destruction of the dorsal motor nucleus did not cause significant degeneration of cardio-inhibitory fibres in the ipsilateral vagus, although this procedure evidently did cause degeneration of fibres subserving gastric secretion (Kerr & Preshaw, 1969). The remaining possibility of their arising solely from the contralateral dorsal motor nucleus was eliminated by Borison & Domjan (1970), who destroyed the

dorsal motor nucleus bilaterally in acute experiments, and found that central ischaemia could still give rise to vagal bradycardia.

While the preceding evidence is convincing that cardio-inhibitory neurones originate outside the dorsal motor nucleus, other methods were necessary to define their location more exactly. We believe that, subject to the points we have discussed, the present results give the best indication of where the cell bodies are to be found; that is, in the lateral part of the nucleus ambiguus, rostral to the obex. However, this evidence was obtained in the cat, and we are aware that there may be differences in other species (Gunn *et al.* 1968), but preliminary observations on the dog (R. M. McAllen & K. M. Spyer, unpublished observations) suggest that their distribution there is similar.

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REFERENCES

AGOSTONI, E., CHINNOCK, J. E., DALY, M. DE B. & MURRAY, J. G. (1957). Functional and histological studies of the vagus nerve and its branches to the heart, lungs and abdominal viscera in the cat. *J. Physiol.* **135**, 182–305.

BIANCHI, A. L. (1971). Localisation et étude des neurones respiratoires bulbaires. *J. Physiol., Paris* **63**, 5–40.

BORISON, H. L. & DOMJAN, D. (1970). Persistence of the cardioinhibitory response to brainstem ischaemia after destruction of the area postrema and the dorsal vagal nuclei. *J. Physiol.* **211**, 263–277.

BRODAL, A. (1969). *Neurological Anatomy in Relation to Clinical Medicine*, 2nd edn. London: Oxford University Press.

BROWN, G. L. & HOLMES, O. (1955). The effects of activity on mammalian nerve fibres of low conduction velocity. *Proc. R. Soc.* B **145**, 1–14.

CAJAL, S. RAMON Y. (1909). *Histologie du Système Nerveux de l'Homme et des Vertébrés* Paris: Maloine.

CALARESU, F. R. & COTTLE, M. K. (1965). Origin of cardiomotor fibres in the dorsal nucleus of the vagus in the cat; a histological study. *J. Physiol.* **176**, 252–260.

CALARESU, F. R. & PEARCE, J. W. (1965). Effects on heart rate of electrical stimulation of medullary vagal structures in the cat. *J. Physiol.* **176**, 241–251.

CHIURUGI, E. & MOLLICA, A. (1954). Contributo alla localizzazione der centro vagale cardioinhibitore. *Archo Fisiol.* **54**, 249 '67.

COOMBS, J. S., CURTIS, D. R. & ECCLES, J. C. (1957). The interpretation of spike potential of motoneurones. *J. Physiol.* **139**, 198–231.

ECCLES, J. C. (1964). *The Physiology of Synapses*. Berlin: Springer-Verlag.

FUSSEY, I. F., KIDD, C. & WHITWAM, J. G. (1973). Activity evoked in the brainstem by stimulation of C fibres in the cervical vagus nerve of the dog. *Brain Res.* **49**, 436–440.

GRUNDFEST, H. (1939). The properties of mammalian B fibres. *Am. J. Physiol.* **127**, 252–262.

GUNN, C. G., SEVELIUS, G., PUIGGARI, M. S. & MYERS, F. K. (1968). Vagal cardiomotor mechanisms in the hindbrain of the dog and cat. *Am. J. Physiol.* **214**, 258–262.

HELLON, R. F. (1971). The marking of electrode tip position in nervous tissue. *J. Physiol.* **214**, 12*P*.

IRIUCHIJIMA, J. & KUMADA, M. (1964). Activity of single vagal fibers efferent to the heart. *Jap. J. Physiol.* **14**, 479–487.

JEWETT, D. L. (1964). Activity of single efferent fibres in the cervical vagus nerve in the dog, with special reference to possible cardio-inhibitory fibres. *J. Physiol.* **175**, 321–357.

KERR, F. W. L. (1967). Function of the dorsal motor nucleus of the vagus. *Science, N.Y.* **157**, 451–452.

KERR, F. W. L. (1969). Preserved vagal visceromotor function following destruction of the dorsal motor nucleus. *J. Physiol.* **202**, 755–769.

KERR, F. W. L. & PRESHAW, R. M. (1969). Secretomotor function of the dorsal motor nucleus of the vagus. *J. Physiol.* **205**, 405–415.

KUNZE, D. L. (1972). Reflex discharge patterns of cardiac vagal efferent fibres. *J. Physiol.* **222**, 1–15.

LAWN, A. M. (1964). The localisation by means of electrical stimulation of the origin and path in the medulla oblongata of the motor nerve fibres of the rabbit oesophagus. *J. Physiol.* **174**, 232–244.

LAWN, A. M. (1966). The nucleus ambiguus of the rabbit. *J. comp. Neurol.* **127**, 307–320.

LIPSKI, J., MCALLEN, R. M. & SPYER, K. M. (1975). The sinus nerve and baroreceptor input to the medulla of the cat. *J. Physiol.* **251**, 61–78.

MCALLEN, R. M. & SPYER, K. M. (1975). The origin of cardiac vagal efferent neurones in the medulla of the cat. *J. Physiol.* **244**, 82–83*P*.

MIDDLETON, S., MIDDLETON, H. H. & GRUNDFEST, H. (1950). Spike potentials and cardiac effects of mammalian vagus nerve. *Am. J. Physiol.* **162**, 553–559.

MILLER, F. R. & BOWMAN, J. T. (1915). The cardio-inhibitory center. *Am. J. Physiol.* **39**, 149–153.

MITCHELL, G. A. G. & WARWICK, R. (1955). The dorsal vagal nucleus. *Acta anat.* **25**, 371–395.

ÖBERG, B. & THOREN, P. (1973). Circulatory responses to stimulation of medullated and non-medullated afferents in the cardiac nerve in the cat. *Acta physiol. scand.* **87**, 121–132.

SALMOIRAGHI, G. C. & BURNS, B. D. (1960). Localisation and patterns of discharge of respiratory neurones in brain-stem of cat. *J. Neurophysiol.* **23**, 2–13.

SZENTAGOTHAI, J. (1952). The general visceral efferent column of the brain stem. *Acta morph. hung.* **2**, 313–328.

THOMAS, M. R. & CALARESU, F. R. (1974). Localization and function of medullary sites mediating vagal bradycardia. *Am. J. Physiol.* **226**, 1344–1349.